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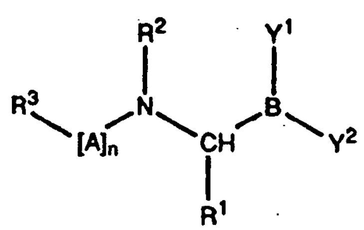
(71) Applicant: THE DU PONT MERCK PHARMACEUTICAL COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).

(72) Inventors: FEVIG, John, Matthew; 987 Church Road, Lincoln University, PA 19352-9349 (US). KETTNER, Charles, Adrian; 2411 Chatham Drive, Wilmington, DE 19803-2709 (US). LEE, Sheng-Lian, O.; 3304 North Rockfield Drive, Wilmington, DE 19810-3221 (US). CARINI, David, John; 1921 Julian Road, Wilmington, DE 19803-3107 (US).

(74) Agents: REINERT, Norbert, F. et al.; The Du Pont Merck Pharmaceutical Company, Legal/Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).

(54) Title: AMIDINO AND GUANIDINO SUBSTITUTED BORONIC ACID INHIBITORS OF TRYPSIN-LIKE ENZYMES



(I)

(57) Abstract

This invention relates to Novel α-aminoboronic acid and corresponding peptide analogs of formula (I) are disclosed.

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Title

Amidino and Guanidino Substituted Boronic Acid Inhibitors of Trypsin-Like Enzymes

Cross Reference to Related Applications

This application is a continuation-in-part of Application Serial Number 08/052,835, filed April 27, 1993.

Field of the Invention

The present invention relates generally to α-aminoboronic acids and corresponding peptide analogs in which the alpha substituent is either an aromatic guanidino, isothiouronium, amidino group, halogen, cyano group or an aliphatic amidino, isothiouronium, or formamidino group.

Background of the Invention

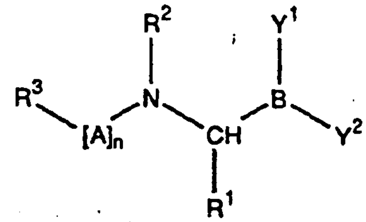
Simple boronic acids are inhibitors of serine proteases. For example, Koehler et al. *Biochemistry* **10**: 2477 (1971) reports that 2-phenylethane boronic acid inhibits chymotrypsin at millimolar levels. The synthesis of boronic acid analogs of N-acyl-α-amino acids has yielded more effective inhibitors. Ac-boroPhe-OH, *R*-1-acetamido-2-phenylethane boronic acid, inhibits chymotrypsin with a K_i of 4 μM Matteson et al. *J. Am. Chem. Soc.* **103**: 5241 (1981). More recently, Shenvi, US 4,537,773 (1985) disclosed that boronic acid analogs of α-amino acids, containing a free amino group, were effective inhibitors of aminopeptidases. Shenvi, US 4,499,082 (1985) discloses that peptides containing an α-aminoboronic acid with a neutral side chain were more effective inhibitors of serine proteases exceeding inhibitors disclosed earlier by as much as 3 orders of magnitude in potency. The chemistry of α-aminoboronic acids was further expanded to the synthesis of peptide

analogs containing boronic acid with positive charged sidechains, boroLysine, boroArginine, boroOrnithine, and isothiouronium analogs (EPA 0 293 881, 12/7/88). This series of compounds have provided highly effective inhibitors of thrombin and other trypsin-like enzymes. The boroArginine analogs specifically designed as thrombin inhibitors are highly effective in the inhibition of blood coagulation both *in vitro* and *in vivo*. In the present invention, this group of compounds is extended to aliphatic amidino and formamidino, to aromatic amidino and guanidino, and to cyano and halogen substituted aromatic boronic acid analogs.

It should be noted that additional boronic acids have been disclosed. Metternich (EP 0471651) have described peptides containing boroArginine and boroLysine which contain at least one unnatural amino acid residue. Elgendy et al. *Tetrahedron Lett.*, **33**, 4209-4212 (1992) have described peptides containing α-aminoboronic acids with aliphatic neutral sidechains which are thrombin inhibitors. Kakkar in (WO 92/07869) has claimed peptide thrombin inhibitors of the general structure, X-Aa_1-Aa_2-NH-CH(Y)-Z where Aa_1 and Aa_2 are unnatural amino acid residues. Z is -CN, -COR, -B(R^2)(R^3), -P(O)(R)(R), and Y is -$[CH_2]_n$-Q or -CH_2-Ar-Q where Q = H, amino, amidino, imidazole, guanidino or isothioureido and n=1-5 and where R_2 and R_3 are the same or different and are selected from the group consisting of OH, OR^6, and NR^6R^7, or R^2 and R^3 taken together represent the residue of a diol. This specialized group of compounds where Z is -B(R^2)(R^3) fall within the scope of our present application. It should be noted that this is a narrow subset of Kakkar et al. However, rather specialized chemical transformations are required to prepare these compounds and Kakkar et al. does not make an enabling disclosure.

Summary of the Invention

A compound of formula (I)



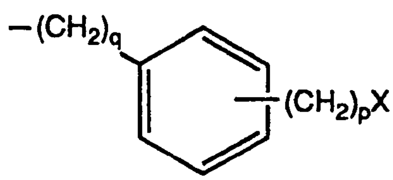
I

wherein

R^1 is

a) C1-C12-alkyl substituted with -CN, -C(NH)NHR^6, -NHC(NH)H, -NHC(NH)NHR^6, -SC(NH)NHR^6, -NHC(NH)NHOH, -NHC(NH)NHCN, -NHC(NH)NHCOR^6, or

b)



;

X is

a) halogen (F, Cl, Br, I)
b) -CN,
c) $-NO_2$,
d) $-CF_3$,
e) $-NH_2$
f) -NHC(NH)H,
g) -NHC(NH)NHOH,
h) -NHC(NH)NHCN,
i) -NHC(NH)NHR^6,
j) -NHC(NH)NHCOR^6,
k) -C(NH)NHR^6,
l) -C(NH)NHCOR^6,
m) -C(O)NHR^2,
n) $-CO_2R^2$,
o) $-OR^2$, or
p) $-OCF_3$

q) $-SC(NH)NHR^6$;

R^2 is

a) H,

b) C1-C4-alkyl,

c) aryl, wherein aryl is phenyl or napthyl optionally substituted with one or two substituents selected from the group consisting of halo (F, Cl, Br, I), C1-C4-alkyl, C1-C4-alkoxy, $-NO_2$, $-CF_3$, $-S(O)_r$-C1-C4-alkyl, -OH, $-NH_2$, -NH(C1-C4-alkyl), $-N(C1\text{-}C4\text{-alkyl})_2$, $-CO_2R^4$, or

d) -C1-C4-alkylaryl, where aryl is defined above;

R^3 is H, alkyl, aryl, alkylaryl, or an NH_2-blocking group comprised of 1-20 carbon atoms;

R^4 and R^5 are independently

a) H,

b) C1-C4-alkyl, or

c) $-CH_2$-aryl, where aryl is defined above;

R^6 is

a) H,

b) C1-C4-alkyl,

c) aryl, wherein aryl is phenyl or napthyl optionally substituted with one or two substituents selected from the group consisting of halo (F, Cl, Br, I), C1-C4-alkyl, C1-C7-alkoxy, $-NO_2$, $-CF_3$, $-S(O)_r$-C1-C4-alkyl, -OH, $-NH_2$, -NH(C1-C4-alkyl), $-N(C1\text{-}C4\text{-alkyl})_2$, $-CO_2R^4$, or

d) -C1-C4-alkylaryl, where aryl is defined above;

A is an amino acid residue or a peptide comprised of 2-20 amino acid residues;

Y^1 and Y^2 are

a) -OH,

b) -F,

c) C1-C8-alkoxy, or

when taken together Y^1 and Y^2 form a

d) cyclic boron ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-3 heteroatoms which can be N, S, or O,

n is 0 or 1;

p is 0 to 3;

q is 0 to 4;

r is 0 to 2;

and pharmaceutically acceptable salts thereof, with the proviso that when R^1 is aliphatic, an R^6 substituent on $-NHC(NH)NHR^6$ cannot be H.

Preferred are those compounds of formula (I) where Y^1 and Y^2 are

a) -OH,

when taken together Y^1 and Y^2 form a

b) cyclic boron pinacol ester, or

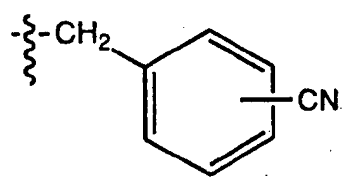
c) cyclic boron pinanediol ester;

R^1 is

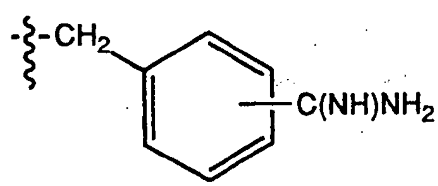
a) $-(CH_2)_3NHC(NH)H$,

b) $-(CH_2)_4C(NH)NH_2$,

c)

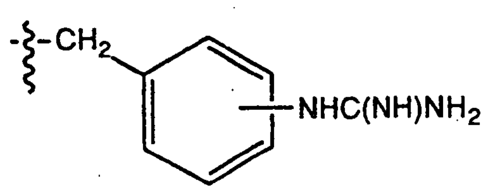


d)



, or

e)



;

R^2 is H;

A is Pro or (D)Phe-Pro;

R^3 is

a) H,

b) Boc,

c) Z, or

d) Ac, or

e) hydrocinnamoyl

f) C1-C10 alkyl sulfonyl

g) C1-C15 alkylaryl sulfonyl

Illustrative of the preferred compounds of this invention are the following:

- Ac-(D)Phe-Pro-NH-CH[$(CH_2)_4$CN]BO_2-$C_{10}H_{16}$
- Ac-(D)Phe-Pro-NHCH[$(CH_2)_4$C(NH)NH_2]BO_2-$C_{10}H_{16}$
- Ac-(D)Phe-Pro-NHCH[$(CH_2)_3$-NHC(NH)H]B$(OH)_2$
- Boc-(D)Phe-Pro-NHCH[$(CH_2)_3$-NHC(NH)H]B$(OH)_2$.
- Ac-(D)Phe-Pro-boroPhe[*m*-C(NH)NH_2]-$C_{10}H_{16}$
- Ac-(D)Phe-Pro-boroPhe(*m*-CH_2NH_2)-$C_{10}H_{16}$
- Ac-(D)Phe-Pro-boroPhe(*m*-Br)-$C_{10}H_{16}$
- Ac-(D)Phe-Pro-boroArg(CN)-$C_{10}H_{16}$
- Ac-(D)Phe-Pro-boroPhe(*p*-CN)-$C_{10}H_{16}$
- Boc-(D)Phe-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- N,N-$(CH_3)_2$-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl (ISOMER I)
- Ac-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl
- Ms-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl
- Boc-(D)Thiazolylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- Boc-(D)3-Pyridylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- Ms-(D)3-Pyridylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- Boc-(D)2-Pyridylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- Boc-(D)2-Thienylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- Ms-(D)2-Thienylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- Boc-(D)Phe-Aze-boroPhe-(m-CN)-$C_{10}H_{16}$
- Hydrocinnamoyl-Pro-boroIrg(CH_3)-OH•HBr

- Ac-(D)Phe-Pro-boroArg(CH_3)-OH•HCl
- $PhCH_2SO_2$-(D)Phe-Pro-boroOrn(CH=NH)-OH•HCl
- $CH_3CH_2CH_2SO_2$-(D)Phe-Pro-boroOrn(CH=NH)-OH•HCl
- $CH_3CH_2CH_2SO_2$-(D)Phe-Pro-boroArg(CH_3)-OH•HCl
- Ac-(D)Phe-Sar-boroOrn(CH=NH)-OH•HCl
- Boc-(D)Phe-Sar-boroPhe(mCN)-$C_{10}H_{16}$
- Boc-(D)Phe-Aze-boroOrn(CH=NH)-OH•HCl
- 4-(Phenyl)benzoyl-boroOrn(CH=NH)-$C_{10}H_{16}$•HCl

This invention also provides compositions comprising one or more of the foregoing compounds and methods of using such compositions in the treatment of aberrant proteolysis such as thrombosis in mammals or as reagents used as anticoagulants in the processing of blood to plasma for diagnostic and other commercial purposes.

Detail Description of the Invention

As used throughout the specifications, the following abbreviations for amino acid residues or amino acids apply:

Ala	=	L-alanine
Arg	=	L-arginine
Asn	=	L-asparagine
Asp	=	L-aspartic acid
Aze	=	azedine-2-carboxlic acid
Cys	=	L-cysteine
Gln	=	L-glutamine
Glu	=	L-glutamic acid
Gly	=	glycine
His	=	L-histidine
HomoLys	=	L-homolysine
Ile	=	L-isoleucine
Irg	=	isothiouronium analog of L-Arg
Leu	=	L-leucine
Lys	=	L-lysine

Met	=	L-methionine
Orn	=	L-ornithine
Phe	=	L-phenylalanine
Pro	=	L-proline
Ser	=	L-serine
Thr	=	L-threonine
Trp	=	L-tryptophan
Tyr	=	L-tyrosine
Val	=	L-valine
Sar	=	L-sarcosine
Phe(4-fluoro)	=	para-fluorophenylalanine

The "D" prefix for the foregoing abbreviations indicates the amino acid is in the D-configuration. "D,L" indicates the amino is present in mixture of the D- and the L-configuration. The prefix "boro" indicates amino acid residues where the carboxyl is replaced by a boronic acid or a boronic acid ester. For example, if R^1 is isopropyl and Y^1 and Y^2 are OH, the C-terminal residue is abbreviated "boroVal-OH" where "-OH" indicates the boronic acid is in the form of the free acid. The pinanediol boronic acid ester and the pinacol boronic acid ester are abbreviated "$-C_{10}H_{16}$" and "$-C_6H_{12}$", respectively. Examples of other useful diols for esterification with the boronic acids are 1,2-ethanediol, 1,3-propanediol, 1,2-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, 5,6-decanediol, and 1,2-dicyclohexylethanediol. The formamidino modified amino group is abbreviated (CH=NH). For example, the formamidino analog of -boroOrn-OH {-NH-CH[$(CH_2)_3$-NH-CH(NH)H]B$(OH)_2$ }is -boroOrn(CH=NH)-OH. Analogs containing sidechain substituents are described by indicating the substituent in parenthesis following the name of the parent residue. For example the analog of boroPhenylalanine containing a *meta* cyano group is -boroPhe(*m*CN)-. N-alkyl substituents on the guanidino

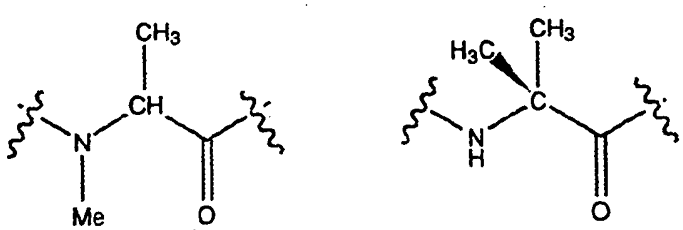
group of boroArg- or on the isothiouronium analogs (boroIrg) are also put in parenthesis in a similar manner. Other abbreviations are: Z, benzyloxycarbonyl; BSA, benzene sulfonic acid; THF, tetrahydrofuran; Boc-, *t*-butoxycarbonyl-; Ac-, acetyl; pNA, *p*-nitro-aniline; DMAP, 4-N,N-dimethylaminopyridine; Tris, Tris(hydroxymethyl)aminomethane; MS, mass spectrometry; FAB/MS, fast atom bombardment mass spectrometry. LRMS(NH_3-CI) and HRMS(NH_3-CI) are low and high resolution mass spectrometry, respectively, using NH_3 as an ion source.

It is understood that many of the compounds of the present invention contain one or more chiral centers and that these stereoisomers may possess distinct physical and biological properties. The present invention comprises all of the stereoisomers or mixtures thereof. If the pure enantiomers or diasteromers are desired, they may be prepared using starting materials with the appropriate stereochemistry, or may be separated from mixtures of undesired stereoisomers by standard techniques, including chiral chromatography and recrystalization of diastereomeric salts.

"NH_2-blocking group" as used herein, refers to various acyl, thioacyl, alkyl, sulfonyl, phosphoryl, and phosphinyl groups comprised of 1 to 20 carbon atoms. Substitutes on these groups maybe either alkyl, aryl, alkylaryl which may contain the heteroatoms, O, S, and N as a substituent or as inchain component. A number of NH_2-blocking groups are recognized by those skilled in the art of organic synthesis. By definition, an NH_2-blocking group may be removable or may remain permanently bound to the NH_2. Examples of suitable groups include formyl, acetyl, benzoyl, trifluoroacetyl, and methoxysuccinyl; alkyl and alkylaryl sulfonyl groups, such as n-propylsulfonyl, phenylmethyl and benzylsulfonyl; aromatic urethane protecting groups,

such as, benzyloxycarbonyl; and aliphatic urethane protecting groups, such as t-butoxycarbonyl or adamantyloxycarbonyl. Gross and Meinhoffer, eds., *The Peptides*, Vol 3; 3-88 (1981), Academic Press, New York, and Greene and Wuts *Protective Groups in Organic Synthesis*, 315-405 (1991), J. Wiley and Sons, Inc., New York disclose numerous suitable amine protecting groups and they are incorporated herein by reference for that purpose.

"Amino acid residues" as used herein, refers to natural or unnatural amino acids of either D- or L-configuration. Natural amino acids residues are Ala, Arg, Asn, Asp, Aze, Cys, Gln, Glu, Gly, His, Ile, Irg Leu, Lys, Met, Orn, Phe, Phe(4-fluoro), Pro, Sar, Ser, Thr, Trp, Tyr, and Val. Roberts and Vellaccio, The Peptides, Vol 5; 341-449 (1983), Academic Press, New York, discloses numerous suitable unnatural amino acids and is incorporated herein by reference for that purpose. Additionally, said reference describes, but does not extensively list, acylic N-alkyl and acyclic α,α-disubstituted amino acids. Included in the scope of the present invention are N-alkyl, aryl, and alkylaryl analogs of both in chain and N-terminal amino acid residues. Similarly, alkyl, aryl, and alkylaryl maybe substituted for the alpha hydrogen. Illustrated below are examples of N-alkyl and alpha alkyl amino acid residues, respectively.



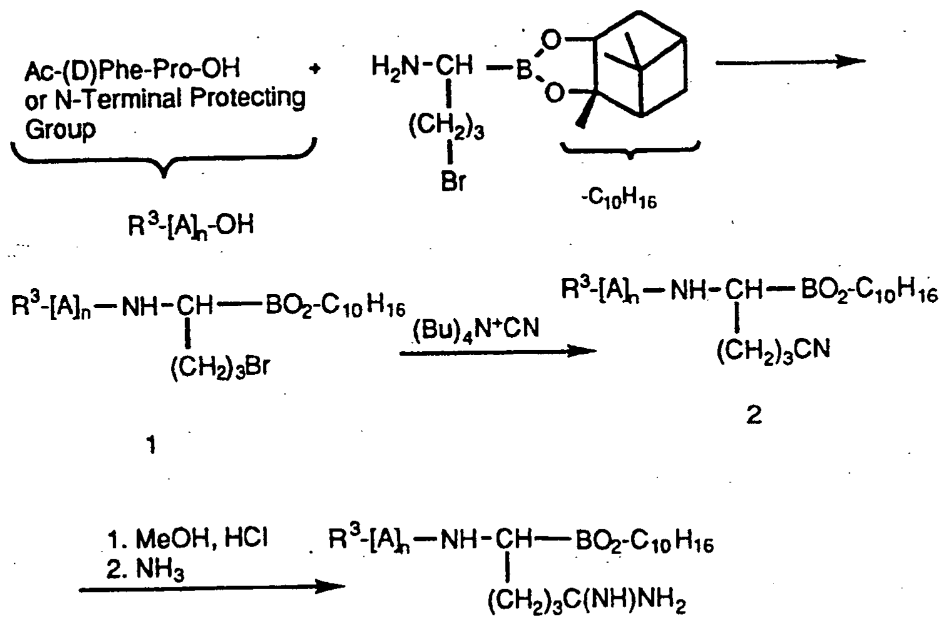
"Amino acids residues" also refers to various amino acids where sidechain functional groups are coupled with appropriate protecting groups known to those skilled in the art. "The Peptides", Vol 3, 3-88 (1981) discloses numerous suitable protecting groups and is incorporated herein by reference for that purpose.

Synthesis

Novel peptide boronic acids containing aliphatic sidechains were prepared by the series of reactions outlined in Scheme I. First, the precursor, NH_2-$CH[(CH_2)_nBr]BO_2$-$C_{10}H_{16}$, n = 3 or 4, was prepared and coupled with an N-terminal protecting group or with an N-terminal and sidechain protected peptide by the procedure we have described previously [Kettner et al. *J. Biol. Chem.* **265** 18289-18297 (1990)]. An example of this product is 1 where the above intermediate is coupled to Ac-(D)Phe-Pro-OH. 1 was converted to the corresponding alkyl cyanide 2 by treatment with tetrabutyl ammonium cyanide in THF at 55 °C for 2 hours. This appears to be a general method for introducing the cyano group. In contrast, other common methods of introducing this group can be applied only with limited success. For example, the reaction of Ac-(D)Phe-Pro-NH-$CH[(CH_2)_4$-$Br]BO_2$-$C_{10}H_{16}$ with KCN in N,N-dimethylformamide failed to yield a detectable product. Our data are consistent with the formation of a cyclic product arising from the nucleophilic displacement of the sidechain bromide by the adjacent amide NH. Treatment of Z-NH-$CH[(CH_2)_4$-$Br]BO_2$-$C_{10}H_{16}$ with NaCN in N,N-dimethylformamide gave the cyano compound, but only in low yield, indicating that cyclization does not occur quite so readily when the urethane protecting group (Z) is present. Typically, 2 was purified by standard techniques such as silica gel chromatography. The corresponding amidine, 3, was prepared by treating the

nitrile with a saturated solution of a mineral acid such as HCl in methanol. Excess solvent and acid were removed by evaporation and the residue was allowed to react with anhydrous ammonia to yield the desired product.

Scheme 1



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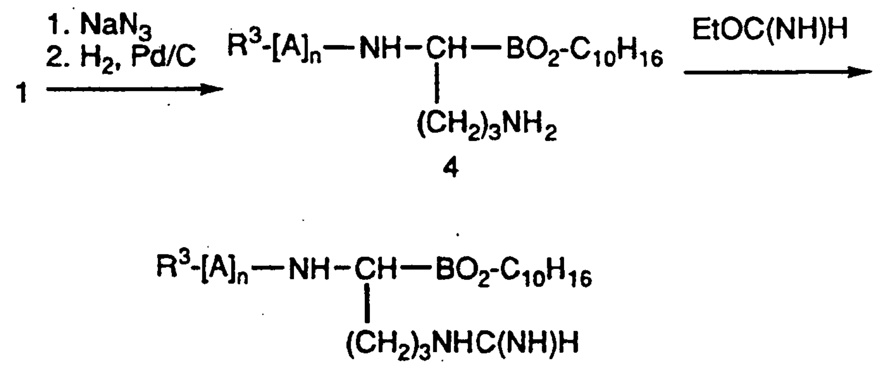
2

3

The formamidino substituted boronic acid, 5, was prepared by the synthesis of the corresponding alkyl amine such as Ac-(D)Phe-Pro-boroOrn-$C_{10}H_{16}$ 4, Scheme 2. This in turn was prepared by treating 1 with sodium azide followed by hydrogenation (Kettner et al., 1990). The amine, 4, was treated with ethyl formimidate to yield the formamidino compound, 5.

Scheme 2



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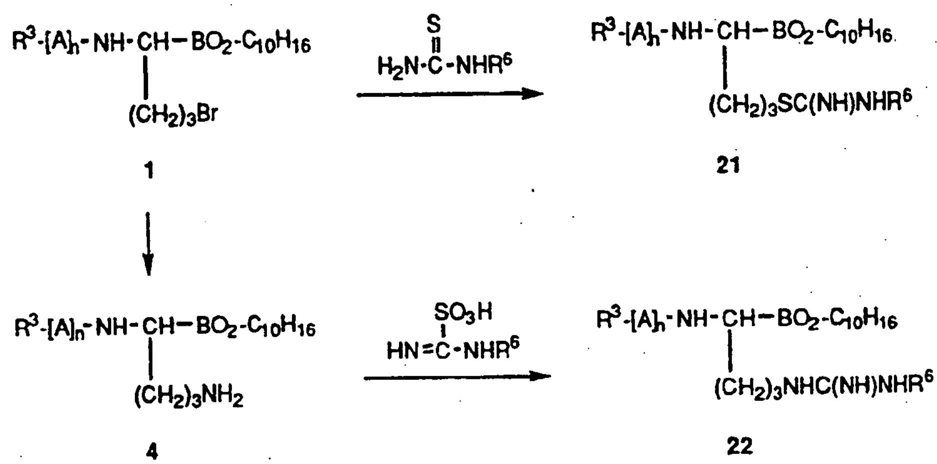
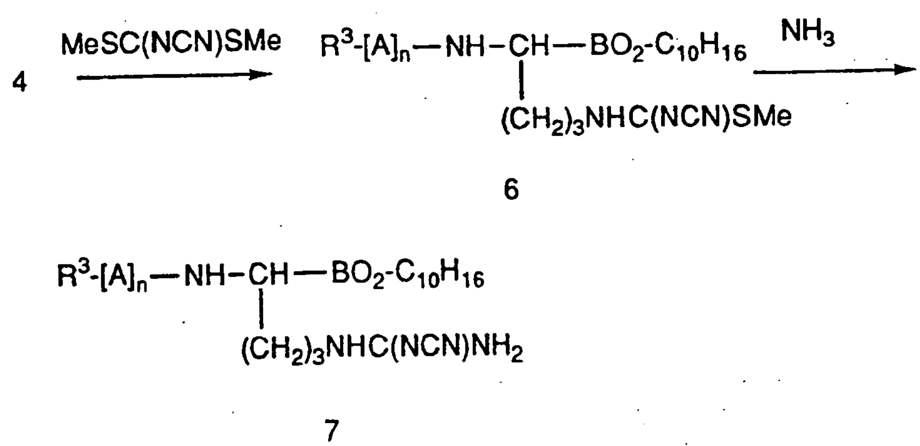
N-substituted isothiouronium derivatives and N-substituted guanidines are readily prepared as shown in Scheme 2a. Treatment of bromide 1 with a thiourea produces directly the isothiouronium 21. Alternatively 1 can be converted to the amine 4 as shown in Scheme 2. Employing a method originally described by Kim et al., *Tetrahedron Lett.* **29**, 3183 (1988), the amine 4 then is heated with a formamidinesulfonic acid in the presence of 4-DMAP to afford the guanidine 22. The required formamidinesulfonic acids can be prepared by oxidation of the corresponding thioureas, see: Walter and Randau, *Liebigs Ann. Chem.* **722**, 98 (1969).

Scheme 2a



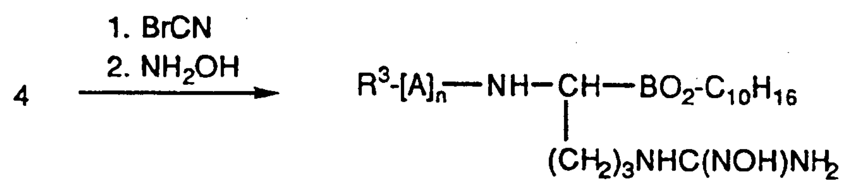
The substituted boronic acid, 7, is prepared by treating 4 with dimethyl cyanodithioiminocarbonate or diphenyl cyanodicarbonimiate to yield the S-methyl isourea (6) or O-phenyl isourea, respectively, using a procedure similar to that reported by Barpill et al. *J. Hereocyclic Chem.* **25**, 1698 (1988), Scheme 3. This intermediate is treated with ammonia in either THF or alcohol to yield the desired product.

Scheme 3



Hydroxyguanidino inhibitors are prepared by treating 4 with cyanogen bromide or cyanogen chloride followed by hydroxylamine to yield 8, Scheme 4. These are known chemical transformations, Nakahara et. al. *Tetrahedron*, **33**, 1591 (1977) and Belzecki et al. *J. Chem. Soc. Chem. Commun.*, 806 (1970).

Scheme 4.



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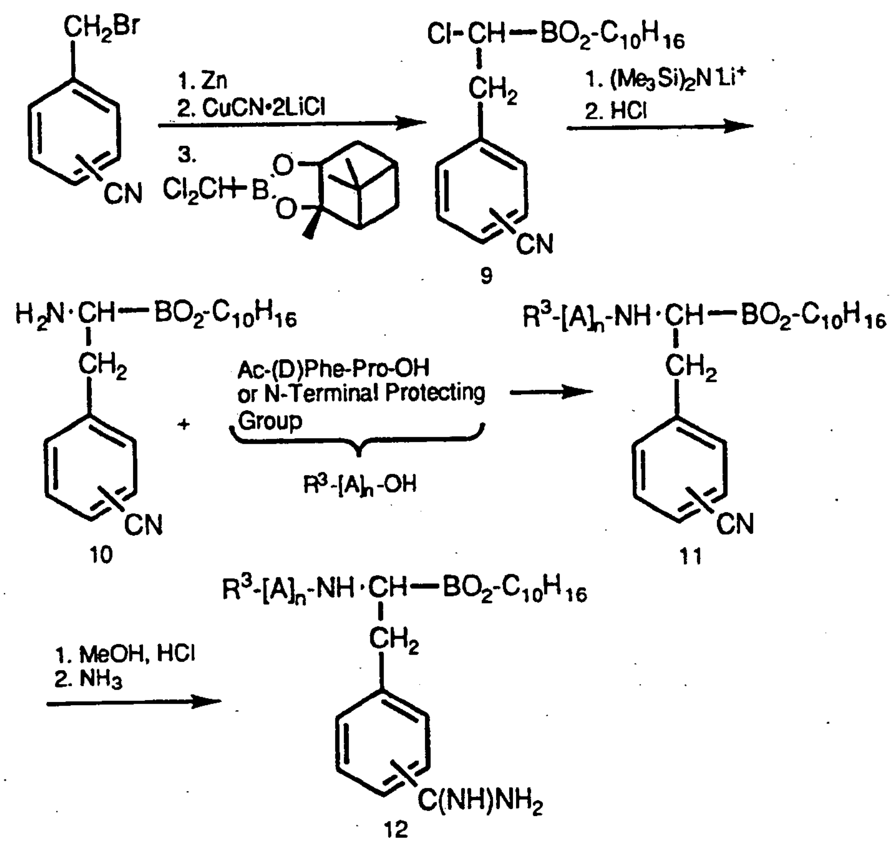
The preparation of new aromatic boronic acids are shown in Scheme 5. Functionalized benzylic anions containing either a halogen or cyano substituent (the cyano group is shown for illustration) are obtained by treatment with activated Zn metal in THF or other inert solvent and then with CuCN·2LiCl [Berk et al. *Organometallics* **9**, 3053-3064 (1990)]. Dichloromethyl boronic acid pinanediol was prepared by the method described by Tsai et al. *Organometallics* **2**, 1543-1545 (1983). It was allowed to react with the transmetalated anion to yield 9. This was the only acceptable method of preparing these functionalized benzylic anions. For example, treatment of *p*-nitobenzyl chloride with lithium metal using the method of Michel et al. *J. Organometallic Chem.* **204**, 1-12 (1981) failed to yield an identifiable product. Similarly, treatment of *p*-cyanobenzyl chloride with lithium naphthalenide in the presence of $ZnCl_2$ using the conditions of Zhu et al. *J. Org. Chem.* **56**, 1445-1453 (1991) did not give the desired product.

The α-aminoboronic acid, 10, was obtained by treating 9 with the lithium salt of hexamethyldisilazane and removing the trimethylsilanyl groups by treatment with anhydrous HCl. 10 was coupled to either an N-terminal protecting group or to a peptide using known techniques.

The aromatic substituted cyanides, 11, were converted to the corresponding amidino compound, 12, using the same sequence of reactions described for preparation of the aliphatic amidino compound, 3.

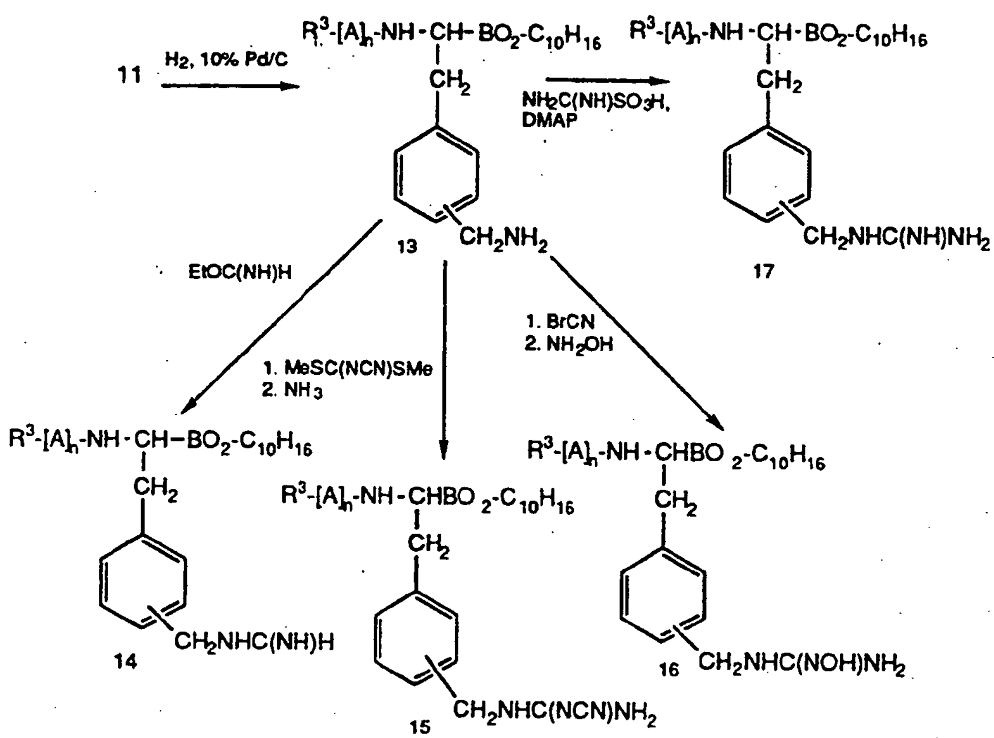
Scheme 5



11 can be hydrogenated to yield the corresponding aminomethyl group as an aromatic substituent 13, Scheme 6. The corresponding formamidino, cyanoguanidino, hydroxyguanidino and guanidino compounds, 14, 15, 16,

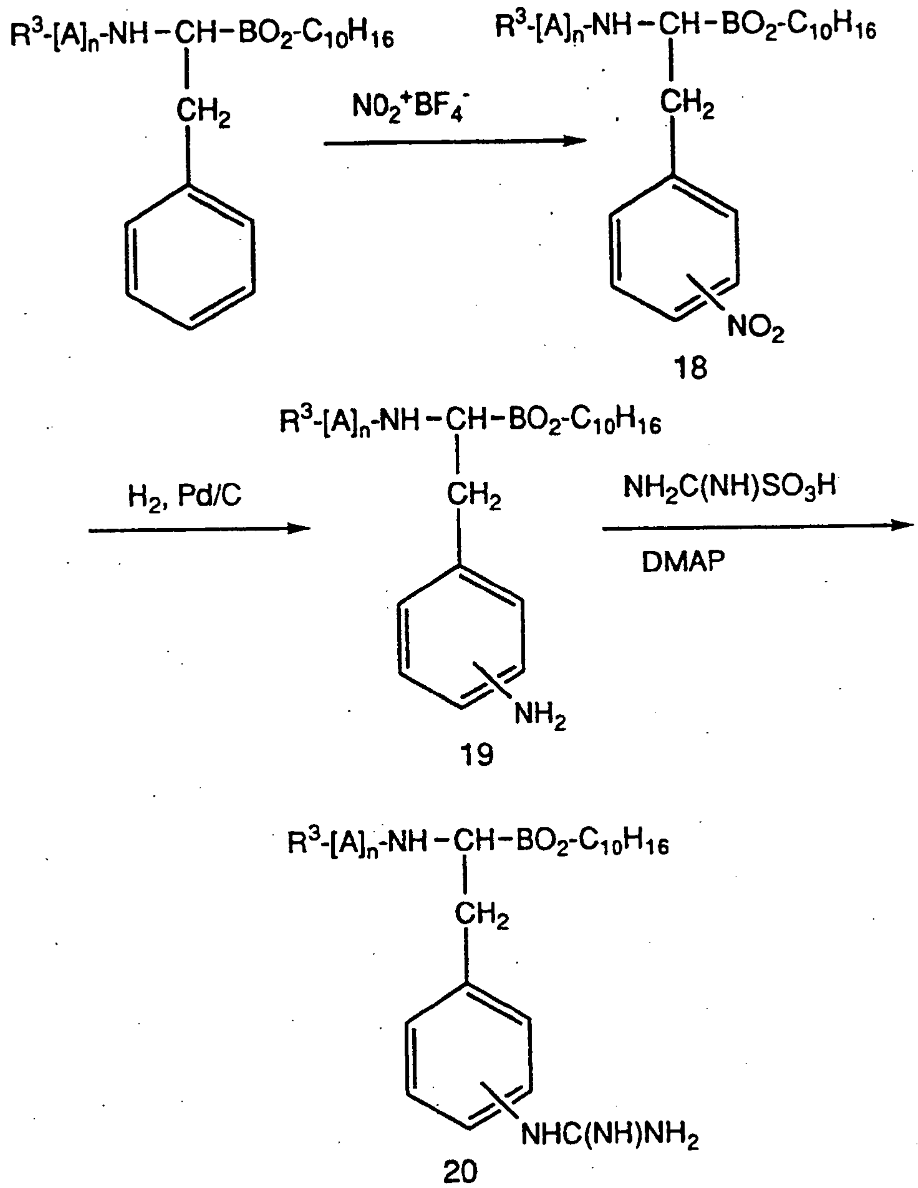
and 17, respectively, are prepared by the procedures described for the aliphatic series, Scheme 1.

Scheme 6



Aromatic guanidino inhibitors, 20, were prepared from precursor R-boroPhe-$C_{10}H_{16}$, Scheme 7. The aromatic ring was nitrated by reaction with $NO^+BF_4^-$ to yield 18 which was reduced to the corresponding amine, 19. The amine is converted to the guanidine by reaction with aminoiminomethane sulfonic acid [Mosher et al. *Tetrahedral Lett.* **29** 3183 (1988)] or cyanamide (Kettner et al. 1990).

Scheme 7



NMR, proton nuclear magnetic resonance, chemical shifts are reported in δ units, parts per million downfield from the internal tetramethylsilane standard. Elemental analyses were conducted by Galbraith Laboratories Inc., Knoxville, TN and Microanalysis Inc., Wilmington, DE. FAB/MS samples of free boronic acids did not give consistent results making it difficult to

monitor the removal of ester protecting groups by this means. However, the presence of the pinanediol and the pinacol groups are readily observed in NMR spectra. For the pinanediol ester, a methyl group is observed at δ 0.9 and the methyl groups of the pinacol groups are observed as singlet at δ 1.1. Following the removal of pinanediol protecting group, MS were run by treating the sample with ~2 equivalents of pinacol in methanol for 5 minutes and evaporating the solvent. Similarly, MS samples of free boronic acid, obtained by removal of the pinacol, were prepared by treating with pinanediol. In some cases, ethylene glycol was used as a matrix for mass spectroscopy to yield the boronic acid-ethyleneglycol ester (designated EG ester). For the subsequent Example see Table 1 for analytical data.

Example 1

Synthesis of Ac-(D)Phe-Pro-NH-CH[$(CH_2)_4CN$]BO_2-$C_{10}H_{16}$

The intermediate, Ac-(D)Phe-Pro-NH-CH[$(CH_2)_4Br$]BO_2-$C_{10}H_{16}$, was prepared using the mixed anhydride procedure. Ac-(D)Phe-Pro-OH (3.04 g, 10 mmol) was dissolved in 50 mL of THF and N-methylmorpholine (1.1 mL, 10 mmol) was added. The solution was cooled to -20°C using a CCl_4 dry ice bath and isobutyl chloroformate (1.30 mL, 10 mmol) was added. After 5 min at -20°C, the mixture was added to NH_2-CH[$(CH_2)_4Br$]BO_2-$C_{10}H_{16}$•HCl (3.81 g, 10 mmol) which was dissolved in 20 mL of THF and precooled to -20°C. Triethylamine (1.39 mL, 10 mmol) was added and the mixture was allowed to stir for 1 h at -20°C and 2 h at room temperature. Insoluble material was removed by filtration and the filtrate was evaporated under a reduced pressure. The residue was dissolved in 50 mL of ethyl acetate and washed subsequently with 75 mL of 0.2 N HCl, 5% $NaHCO_3$, and saturated aqueous sodium chloride. The organic phase was dried over Na_2SO_4 and concentrated *in vacuo* to give

Ac-(D)Phe-Pro-NHCH[$(CH_2)_4$Br]BO_2-$C_{10}H_{16}$ (6.01 g, 95% yield).

The bromide (1.89 g, 3.0 mmol) and tetra-n-butyl ammonium cyanide (3.2 g, 11.8 mmol, 4 eq) were dissolved in 50 mL of acetonitrile. This solution was heated at 90°C for 3 h and solvent was removed under reduced pressure. The residue was dissolved in 50 mL of ethyl acetate and was washed with three 50 mL portions of saturated aqueous NaCl. The ethyl acetate solution was dried over anhydrous Na_2SO_4 and evaporated to give 2.5 g of crude product. It was purified by silica gel chromatography using 5% MeOH in $CHCl_3$ as an eluent to yield the desired product (0.50 g, 29% yield). LRMS (NH_3-CI) m/e calcd. for M ($C_{32}H_{45}N_4O_5B$) + NH_4^+: 594.4. Found: 594. HRMS(NH_3-CI) m/e calcd. for M ($C_{32}H_{45}N_4O_5B$) + H^+: 577.3561. Found: 577.3555.

Example 2

Synthesis of Ac-(D)Phe-Pro-NHCH[$(CH_2)_4$C(NH)NH_2]-BO_2-$C_{10}H_{16}$•benzene sulfonic acid

The nitrile, (Example 1, 0.40 g, 0.70 mmol), was dissolved in 50 mL of a cold solution of saturated HCl in methanol and the solution was stirred overnight at 4°C. The solution was then concentrated under reduced pressure. The residue was dissolved in anhydrous methanol (50 mL), gaseous NH_3 was bubbled through the solution for 1 h, and the solution was heated at 50 °C for 3 h. Solvent was evaporated, the residue was suspended in minimum volume of methanol, and 0.11 g of benzenesulfonic acid (1 eq) was added. Methanol was evaporated and the residue was triturated with hexane to yield the desired product as a pale yellow powder (0.52 g, 99 % yield).

FABMS: m/e calculated for M ($C_{32}H_{48}N_5O_5B$) + H^+: 594.38. Found: 594.14. HRMS(NH_3-CI) m/e calcd for M ($C_{32}H_{48}N_5O_5B$) + H^+: 594.3827. Found: 594.3824.

Example 3

<u>Synthesis of Ac-(D)Phe-Pro-NHCH[$(CH_2)_3$NHC(NH)H]BO_2-$C_{10}H_{16}$ or Ac-(D)Phe-Pro-boroOrn(CH=NH)-$C_{10}H_{16}$</u>

Ethyl formimidate•HCl was prepared by the procedure of Ohme and Schmitz *Angew. Chem. Internat. Edit.* **6** 566 (1967) and Ac-(D)Phe-Pro-boroOrn-$C_{10}H_{16}$ was prepared by the procedure of Kettner et al. (1990). The formimidate (1.29 g, 11.7 mmol) and 4-N,N-dimethylaminopyridine (1.44 g) were added to a solution of Ac-(D)Phe-Pro-boroOrn-$C_{10}H_{16}$•BSA (2.78 g, 3.92 mmol) dissolved in 40 mL of ethanol. The resulting solution was refluxed for 8 h. After removal of solvent, the residue was purified by chromatography using a column of Sephedex™LH 20 and methanol as a solvent to give pure product (1.28 g, 56 % yield).

HRMS(NH_3-CI) m/e calcd. for M ($C_{31}H_{46}BN_5O_5$) + H^+: 580.3670. Found: 580.3679.

Example 4

<u>Synthesis of Ac-(D)Phe-Pro-NHCH[$(CH_2)_3$-NHC(NH)H]$B(OH)_2$</u>

The pinanediol protecting group on the boronic acid portion of Ac-(D)Phe-Pro-NHCH[$(CH_2)_3$-NHC(NH)H]-BO_2-$C_{10}H_{16}$•HCl (Example 3) was removed by transesterification using the procedure we have described previously in U.S.Application 08/010731. The pinanediol ester (0.30 g, 0.51 mmol) and phenyl boronic acid (0.31 g, 2.6 mmol) were suspended in 10 mL of a 1: 1 mixture of ether and water and was allowed to stir for 2.5 h at room temperature. The phases were separated and the aqueous phase was extensively washed with ether. The aqueous phase was evaporated to yield a solid. This material was triturated with ether to give the desired product as an amorphous white solid, 0.20 g (83 % yield). LRMS (NH_3-CI) m/e calcd. for the pinacol ester M ($C_{27}H_{42}N_5O_5B$) + H^+: 528.3. Found: 528. HRMS (NH_3-

CI) m/e calcd. for the pinacol ester M ($C_{27}H_{42}N_5O_5B$) + H^+: 528.3357. Found: 528.3347.

Example 5

Synthesis of Boc-Pro-NHCH[(CH_2)$_3$NHC(NH)H]BO_2-$C_{10}H_{16}$

Boc-Pro-boroOrn-$C_{10}H_{16}$•BSA was also prepared by the procedure described previously (Kettner et al. 1990). This peptide (3.0 g, 6.5 mmol) was dissolved in 25 mL of absolute ethanol, 4-N,N-dimethylaminopyridine (1.6 g, 12.9 mmol) and ethyl formimidate•HCl (1.4 g, 12.9 mmol) were added. The solution was heated on a 85 °C oil bath for 1 h. Solvent was evaporated and the residue was dissolved in methanol and was chromatogramed on a 2.5 X 100 cm column of LH20 in methanol to yield 1.3 g of the desired product.

LRMS (NH_3-CI) m/e calcd. for M ($C_{25}H_{43}N_4O_5B$) + H^+: 491.5. Found: 491.

Example 6

Synthesis of Boc-(D)Phe-Pro-NHCH[(CH_2)$_3$-NHC(NH)H]BO_2-$C_{10}H_{16}$

The reaction was run using the procedure described for Example 3. Boc-(D)Phe-Pro-boroOrn-$C_{10}H_{16}$•BSA (3.7 g, 4.78 mmol), 4-N,N-dimethylaminopyridine (1.71 g, 13.8 mmol), and ethyl formimidate•HCl (1.54 g, 13.8 mmol) were dissolved in 50 mL of absolute ethanol and was heated at 85 °C for 7 h. The desired product was obtained by chromatography on a column of LH 20 in a yield of 1.56 g.

HRMS (NH_3-CI) m/e calcd for M ($C_{34}H_{52}N_5O_6B$) + H^+: 638.4089. Found: 638.4082.

Example 7

Synthesis of Boc-(D)Phe-Pro-NHCH[(CH_2)$_3$-NHC(NH)H]B(OH)$_2$. Boc-(D)Phe-Pro-NHCH[(CH_2)$_3$-NHC(NH)H]BO_2-$C_{10}H_{16}$• 0.40 BSA, 0.60 HCl (Example 6, 0.16

g, 0.22 mmol) and phenyl boronic acid (0.13g, 1.1 mmol) were placed in mixture of 5 mL of ether and 5 mL of water and was allowed to stir for 4 h at room temperature. The phases were separated and the organic phase was washed with 5 mL of water. The combined aqueous phases were extensively washed with ether. The aqueous phase was evaporated and the residue triturated with ether to yield the desired product as a white solid, 0.10 g. LRMS (NH_3-CI) m/e calcd. for the pinacol ester M ($C_{30}H_{48}N_5O_6B$) + H^+: 586.4. Found: 586. HRMS (NH_3-CI) m/e calcd. for the pinacol ester M ($C_{30}H_{48}N_5O_6B$) + H^+: 586.3776. Found: 586.3772.

Example 8

Synthesis of H-(D)Phe-Pro-NHCH[$(CH_2)_3$-NHC(NH)H]BO_2-$C_{10}H_{16}$•2HCl

Boc-(D)Phe-Pro-NHCH[$(CH_2)_3$-NHC(NH)H]BO_2-$C_{10}H_{16}$•0.40 BSA, 0.60 HCl (Example 6, 0.20 g, 0.25 mmol) was dissolved in 2 mL of 4 N HCl: dioxane and was allowed to stir for 1 h at room temperature. Solvent was evaporated and the residue was triturated with ether to yield 0.18 g of the desired product.

HRMS (NH3-CI) m/e calcd for M ($C_{29}H_{44}N_5O_4B$) + H^+: 538.3565. Found: 538.3569.

Example 9

Synthesis of H-(D)Phe-Pro-NHCH[$(CH_2)_3$-NHC(NH)H]$B(OH)_2$

H-(D)Phe-Pro-NH-CH[$(CH_2)_3$-NH-C(NH)H]BO_2-$C_{10}H_{16}$•0.35 BSA, 0.65 HCl (Example 8, 0.10 g, 0.16 mmol) was allowed to react with phenyl boronic acid according to the procedure in Example 4 to yield the desired product, 0.053 g. LRMS (NH_3-CI) m/e calcd. for the pinacol ester M ($C_{25}H_{40}N_5O_4B$) + H^+: 486.3. Found: 486. HRMS (NH_3-CI) m/e calcd for pinacol ester M ($C_{25}H_{40}N_5O_4B$) + H^+: 486.3251. Found: 486.3255.

Example 10

Synthesis of $H_2NCH[CH_2C_6H_4$-*m*-$CN]BO_2C_{10}H_{16}$ •HCl or H-boroPhe(*m*-CN)-$C_{10}H_{16}$•HCl

The first intermediate, Cl-CH[CH_2-(*m*-cyanophenyl)]BO_2-$C_{10}H_{16}$, was prepared from *m*-cyanobenzyl bromide and dichloromethyl boronate pinanediol. Zinc dust (1.0 g) in 1 mL of THF was cooled to 0-5°C and a solution of *m*-cyanobenzyl bromide (1.37 g, 7.0 mmol) in 7 mL of THF was added dropwise (5 sec/drop). The reaction mixture was allowed to stir at 5°C for 2 h. A mixture consisting of LiBr (1.22 g, 14 mmol), CuCN (0.63 g, 7.0 mmol), and 6 mL of THF was placed in a 50 ml flask and cooled to -40°C; then the benzylic organozinc reagent was added by cannulation. The mixture was allowed to warm to -20°C and stir for 5 min. It was cooled to -78°C and neat dichloromethyl boronic acid pinanediol (1.47 g, 5.6 mmol) was added dropwise. The resulting mixture was stirred at -78°C for 2 h and at room temperature for 2 days. Saturated aqueous NH_4Cl (20 mL) was added to the mixture and the aqueous solution was extracted with three 20 ml portions of ether. The combined organic layers was dried over anhydrous $MgSO_4$ and evaporated *in vacuo* to give crude compound (1.8 g). It was purified by silica gel chromatography where the column was stepwise eluted with hexane (100 mL) and then 15% ether in hexane (200 mL) to give the desired product 0.53 g (27% yield). LRMS(NH_3-CI) m/e calcd. for M ($C_{19}H_{23}NO_2BCl$)+NH_4^+: 361.2. Found: 361.1.

To a solution of hexamethyldisilazane (0.21 mL, 0.98 mmol) in 2 mL of THF at -78°C was added *n*-butyllithium (1.45 M, 0.67 mL, 0.98 mmol). The solution was allowed to slowly warm to room temperature to ensure the anion generation was complete. The resulting solution was then cooled to -78°C and Cl-CH[CH_2-(*m*-cyanophenyl)]BO_2-$C_{10}H_{16}$ (0.33 g, 0.98 mmol) in 2 mL of

THF was added. The mixture was allowed to warm to room temperature and to stir overnight. Solvent was evaporated and 8 mL of hexane was added to give a suspension. HCl in dioxane (4.1 N, 1.5 mL, 6.0 mmol) was added at -78°C. The mixture was slowly warmed to room temperature and stirred for 2 h. Additional hexane (6 mL) was added and crude product was isolated as a precipitate. This product was dissolved in chloroform and insoluble material was removed by filtration. The filtrate was evaporated at a reduced pressure to give an oil (~0.2 g). Final purification was achieved by chromatography on a column of Sephedex™ LH 20 column using methanol as a solvent. H-boroPhe(*m*-CN)-$C_{10}H_{16}$•HCl was obtained as an oil (0.12 g, 34% yield). HRMS(NH_3-CI) m/e calcd. for M ($C_{19}H_{26}BN_2O_2$) + H^+: 325.2087. Found: 325.2094.

Example 11

Synthesis of Ac-(D)Phe-Pro-boroPhe(*m*-CN)-$C_{10}H_{16}$

Ac-(D)Phe-Pro-OH (0.10 g, 0.33 mmol) and N-methylmorpholine (0.037 mL, 0.33 mmol) were allowed to react with isobutyl chloroformate (0.043 mL, 0.33 mmol) in 5 mL of THF at -20°C. After 5 min, H-boroPhe(*m*-CN)-$C_{10}H_{16}$•HCl, (Example 10, 0.12 g, 0.33 mmol) dissolved in 3 mL of cold THF and triethylamine (0.046 mL, 0.33 mmol) were added. The mixture was allowed to stir at -20°C for 1 h and to stir at room temperature for an additional hour. Insoluble material was removed by filtration and solvent was evaporated. The residue was dissolved in ethyl acetate and was washed with 0.20 N HCl, 5 % $NaHCO_3$, and saturated aqueous NaCl. The organic layer was dried over anhydrous Na_2SO_4 and was evaporated *in vacuo* to give 0.2 g of an oil. It was purified by chromatography on a column of Sephedex™ LH 20 yielding 0.13 g of desired product (65% yield).

HRMS(NH_3-CI) m/e calcd. for M ($C_{35}H_{43}BN_4O_5$) + H^+: 611.3405. Found: 611.3416.

Example 12

Synthesis of Ac-(D)Phe-Pro-boroPhe[*m*-C(NH)NH_2]-$C_{10}H_{16}$

Ac-(D)Phe-Pro-boroPhe(*m*-CN)-$C_{10}H_{16}$, Example 11, (50 mg) was dissolved in 5 mL of saturated solution of HCl in methanol. The solution was allowed to stir overnight at 4 °C. After removal of solvent, the residue was resuspended in 5 mL of anhydrous methanol, cooled to 0°C, and anhydrous NH_3 was bubbled through the solution for 0.5 h. It was heated at 60°C for 6.2 h. Solvent was evaporated and one equivalent of benzene sulfonic acid (13 mg) and 1 mL of methanol were added. Solvent was evaporated under N_2 and the product was triturated with ether to give the desired product as a pale brown powder (65 mg, 100% yield). HRMS(NH_3-CI) m/e calcd. for M ($C_{35}H_{47}BN_5O_5$) + H^+: 628.3670. Found: 628.3688.

Example 13

Synthesis of Ac-(D)Phe-Pro-boroPhe(*m*-CH_2NH_2)-$C_{10}H_{16}$

Ac-(D)Phe-Pro-boroPhe(*m*-CN)-$C_{10}H_{16}$ was placed in 5 mL of methanol, 10% Pd/C (25 mg) and 0.1N HCl (0.41 mL) were added, and the mixture was stir under H_2 at room temperature for 2.5 h. The solution was filtered through Celite and washed with 20 mL of methanol. The filtrate was concentrated under a reduced pressure and the residue was triturated with ether to give pure product as white powder (15.6 mg, 59% yield). HRMS(NH_3-CI) m/e calcd. for M ($C_{35}H_{47}N_4O_5B$) + H^+: 615.3718. Found: 615.3700.

Example 14

Synthesis of Ac-(D)Phe-Pro-boroPhe(*m*-Br)-$C_{10}H_{16}$

Cl-CH[CH_2-(*m*-bromo-phenyl)]BO_2-$C_{10}H_{16}$ was prepared making the anion of *m*-bromobenzyl bromide and coupling

it to dichloromethyl boronic acid pinanediol. This intermediate and the corresponding amine were prepared using the procedure described for Example 10. The amine was coupled to Ac-(D)Phe-Pro-OH using the method described in Example 11.

LRMS(NH_3-CI) m/e calcd. for M ($C_{34}H_{43}N_3O_5BrB$) + H^+: 666.3. Found: 666.2.

Example 15

Synthesis of Ac-(D)Phe-Pro-boroArg(CN)-$C_{10}H_{16}$

Ac-(D)Phe-Pro-boroOrn-$C_{10}H_{16}$•HCl (0.15 g, 0.25 mmol), triethylamine (0.035 mL, 0.25 mmol), and diphenyl cyanocarbonimidate (Aldrich, 0.060 g, 0.25 mmol) were heated at a gentle reflux for 5 h in THF and then stirred overnight at room temperature. The sample was diluted with chloroform and washed with water and saturated aqueous NaCl. It was dried over K_2CO_3 and purified by silica gel chromatgraphy using methanol: chloroform (1:9) as a solvent to yield 80 mg of Ac-(D)Phe-Pro-NH-CH[(CH_2)$_3$-NH-C(N-CN)O-Ph]BO_2-$C_{10}H_{16}$. LRMS(NH_3-CI) m/e calcd. for M ($C_{38}H_{49}N_6O_6B$) + H^+: 697.7. Found: 697.

The above product (0.060 g, 0.080 mmol) was dissolved in 0.5 mL of THF and was allowed to react with 1 equivalent of 30% aqueous ammonia for 30 min at room temperature. Four additional equivalent of ammonia were added and the solution was allowed to stir overnight at room temperature. A large excess of ammonia was added and the reaction mixture was allowed to stir 2 days at room temperature. The reaction mixture was diluted with methylene chloride and was washed with water and saturated aqueous NaCl. It was dried over K_2CO_3 and purified by chromatography on a silica gel column using methanol and chloroform (1:9) as a solvent to yield 15 mg of the desired product. LRMS(NH_3-CI) m/e calcd. for M ($C_{32}H_{46}N_7O_5B$) + H^+: 619.5. Found: 620.

Example 16

Synthesis of Ac-(D)Phe-Pho-boroPhe(*p*-CN)-$C_{10}H_{16}$

$ClCH[CH_2C_6H_4$-*p*-$CN]BO_2C_{10}H_{16}$ was prepared by making the anion of *p*-cyanobenzyl bromide and coupling it to dichloromethyl boronate pinanediol. This intermediate and the corresponding amine were prepared using the procedure described for Example 10. $NH_2CH[CH_2C_6H_4$-*p*-$CN]BO_2C_{10}H_{16}$ (Example 78) was coupled to Ac-(D)Phe-Pro-OH using the method described in Example 11.

HRMS (NH_3-Cl)m/e calcd. for M ($C_{35}H_{43}N_4O_5B$) + H^+: 611.3405. Found: 611.3408.

Example 17

Synthesis of Boc-(D)Phe-Pro-boroPhe(*m*CN)-$C_{10}H_{16}$

Boc-(D)Phe-Pro-boroPhe(*m*CN)-$C_{10}H_{16}$ was prepared by reacting Boc-(D)Phe-Pro-OH (0.43 g, 1.2 mmol), H-boroPhe(*m*CN)-$C_{10}H_{16}$•HCl (0.42 g, 1.2 mmol), N-methylmorpholine (0.26 mL, 2.4 mmol), hydroxybenzotriazole•H_2O (0.36 g, 2.4 mmol), and dicyclohexylcarbodiimide (0.25 g, 1.2 mmol) in 20 mL of dichloromethane overnight at room temperature. The reaction mixture was filtered and the filtrate was chromatogramed on a 2.5 X 100 cm column of Sephedex LH-20 in methanol to yield 0.36 g of the desired product.

Example 18

Synthesis of H-(D)Phe-Pro-boroPhe(*m*CN)-$C_{10}H_{16}$•HCl

Boc-(D)Phe-Pro-boroPhe(*m*CN)-$C_{10}H_{16}$ (0.21 g) was allowed to react with 2 mL of 4 N HCl dioxane for 2 h at room temperature. Solvent was removed by evaporation and the residue was triturated with ether to yield 0.11 g of the desired product as a white solid.

Example 19

Synthesis of H-(D)Phe-Pro-boroPhe(*m*CN)-OH•HCl

H-(D)Phe-Pro-boroPhe(*m*CN)-$C_{10}H_{16}$•HCl (0.63 g, 1.0 mmol) was allowed to react with 5 equivalents of phenylboronic acid using the procedure described for Example 7 to yield 0.46 g of product.

Example 20

Synthesis of N,N Dimethyl-(D)Phe-Pro-boroPhe(*m*CN)-OH•HCl

H-(D)Phe-Pro-boroPhe(*m*CN)-OH•HCl (0.20 g, 0.42 mmol), 37% aqueous formaldehyde (0.34 mL, 4.2 mmol) were dissolved in 2 mL of acetonitrile. Sodium cyanoborohydride (0.080 g, 1.3 mmol) was added and after 5 min glacial acetic acid (20μL) were added. The reaction pH was ~7. After 5 h, additional acetic acid (20 μL) were added and the mixture was stirred for 1 h. The reaction mixture was poured into 20 mL of ethyl acetate and the organic phase was washed with 10 mL of saturated aqueous sodium chloride and dried over anhydrous sodium sulfate. Evaporation of solvent yielded 0.16 g of an oil which was triturated with ether to give a white solid.

Example 52

Synthesis of Ac-(D)Phe-Pro-NH-H[$(CH_2)_3$SC(NH)$NHCH_3$]$B(OH)_2$

The intermediate, Ac-(D)Phe-Pro-NH-CH[$(CH_2)_3Br$]$BO_2C_{10}H_{16}$, was prepared using the mixed anhydride procedure of example 1. A solution of this bromide (0.35 g, 0.57 mmol) and 1-methyl-2-thiourea (0.077 g, 0.85 mmol) in 10 mL of absolute ethanol was refluxed for 18 hours. After cooling the solvent was removed under vacuum, and the product was separated from excess thiourea employing chromatography (elution: methanol) on Sephadex® LH-20 gel to provide 0.31 g (77%) of the isothiouronium product. This boronic acid ester (0.28 g) was then deprotected as described in example 4 to afford 0.13 g (57%) of the desired product. LRMS (ESI) m/e calcd. for M ($C_{22}H_{34}BN_5O_5S$) + H^+: 492. Found:

492. HRMS (NH_3-CI) m/e calcd. for ethylene glycol ester M ($C_{24}H_{36}BN_5O_5S$) + H+: 518.260847. Found: 518.261656.

Example 54

Synthesis of Ac-(D)Phe-Pro-NH-CH[(CH_2)$_3$NHC(NH)$NHCH_3$]-B$(OH)_2$

A solution of Ac-(D)Phe-Pro-boroOrn-$BO_2C10H16$ • HCl [0.50 g, 0.85 mmol, prepared by the procedure of Kettner et al.(1990)], 4-methylaminopyridine (0.21 g, 1.7 mmol), N-methylamino-iminomethanesulfonic acid (0.24 g, 1.7 mmol), and 10 mL of absolute ethanol was refluxed for 18 hours. After cooling the mixture was filtered and the precipitate was washed with chloroform. The combined filtrates were concentrated under vacuum, and the residue was dissolved in 10 mL of chloroform. The chloroform solution was washed with ice-cold 0.1 N hydrochloric acid (2 X 3 mL), ice-cold water (2 X 3 mL), and brine. The resulting organic solution was then dried over anhydrous magnesium sulfate, filtered, and concentrated. The product was purified employing chromatography (elution: methanol) on Sephadex® LH-20 gel to provide 0.30 g (55%) of the guanidine. This boronic acid ester was then deprotected as described in example 4 to afford 0.14 g (59%) of the desired product. LRMS (NH_3-CI) m/e calcd. for ethylene glycol ester M ($C_{24}H_{37}BN_6O_5$) + H^+: 501. Found: 501. HRMS (NH_3-CI) m/e calcd. for ethylene glycol ester M ($C_{24}H_{37}BN_6O_5$) + H+: 501.299674. Found: 501.300760.

The examples of Table 1 can be prepared by the schemes and procedures described above using the appropriate starting materials.

Table 1.

EX #	Compound	MS Method	LRMS CALC'D	LRMS FOUND
1	Ac-(D)Phe-Pro-NH-CH[$(CH_2)_4$CN]$BO_2C_{10}H_{16}$	NH_3/CI (M+NH4)	594.4	594
2	Ac-(D)Phe-Pro-NH-CH[$(CH_2)_4$-C(NH)NH_2]$BO_2C_{10}H_{16}$•BSA	NH_3/CI (M+H)	594.4	594
3	Ac-(D)Phe-Pro-boroOrn(CH=NH)]-$C_{10}H_{16}$•HCl	NH_3/CI (M+H)	580.4	580
4	Ac-(D)Phe-Pro-boroOrn(CH=NH)]-OH•HCl	NH_3/CI pinacol ester+H	528.3	528
5	Boc-Pro-boroOrn(CH=NH)-$C_{10}H_{16}$•HCl	NH_3/CI (M+H)	491.5	491
6	Boc-(D)Phe-Pro-boroOrn(CH=NH)]-$C_{10}H_{16}$•0.5 HCl•0.5 BSA	NH_3/CI (M+H)	638.4	638
7	Boc-(D)Phe-Pro-boroOrn(CH=NH)]-OH•0.6 HCl•0.4 BSA	NH_3/CI pinacol ester+H	586.4	586
8	H-(D)Phe-Pro-boroOrn(CH=NH)]-$C_{10}H_{16}$•0.5 HCl•0.5 BSA	NH_3/CI (M+H)	538.4	538
9	H-(D)Phe-Pro-boroOrn(CH=NH)]-OH•0.65 HCl•0.35 BSA	NH_3/CI pinacol ester+H	486.3	486
10	H-boroPhe(mCN)-$C_{10}H_{16}$•HCl			
11	Ac-(D)Phe-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+H)	611.3	611
12	Ac-(D)Phe-Pro-boroPhe-(m-C(NH)NH_2)-$C_{10}H_{16}$•BSA	NH_3/CI (M+H)	628.4	628
13	Ac-(D)Phe-Pro-boroPhe-(m-CH2NH_2)-$C_{10}H_{16}$•HCl	NH_3/CI (M+H)	615.4	615
14	Ac-(D)Phe-Pro-boroPhe-(m-Br)-$C_{10}H_{16}$	NH_3/CI (M+NH4)	683.4	683
15	Ac-(D)Phe-Pro-boroArg(CN)-$C_{10}H_{16}$•HCl	NH_3/CI (M+H)	619.5	620
16	Ac-(D)Phe-Pro-boroPhe-(p-CN)-$C_{10}H_{16}$	NH_3/CI (M+NH4)	628.4	628
17	Boc-(D)Phe-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+NH4)	686.4	686

18	H-(D)Phe-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$•HCl	NH_3/CI (M+H)	569.3	569
19	H-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl	NH_3/CI EG ester+H	461.2	461
20	N,N-$(CH3)_2$-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl (ISOMER I)	NH_3/CI EG ester+H	489.3	489
21	Ac-(D)Phe-Pro-boroPhe-(p-CH_2NH_2)-$C_{10}H_{16}$• BSA	NH_3/CI (M+H)	615.4	615
22	Ac-(D)Phe-Pro-boroPhe-(p-C(NH)NH_2)-$C_{10}H_{16}$• BSA	FAB (M+H)	628.37	628.44
23	Ac-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl	NH_3/CI EG ester+NH4	520.3	52[illegible]
24	Ms-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl	NH_3/CI EG ester+NH4	556.2	556
25	N-CH_3-(D)Phe-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$•HCl	NH_3/CI (M+H)	583.4	583.3
26	H-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$•HCl	NH_3/CI (M+H)	422.3	422
27	Boc-(D)Thiazolylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+H)	676.4	676.4
28	Boc-(D)3-Pyridylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+H)	670.4	670.4
29	H-(D)Thiazolylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$•HCl	NH_3/CI (M+H)	576.3	57[illegible]
30	H-(D)3-Pyridylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$ •HCl	NH_3/CI (M+H)	570.3	570
31	Ms-(D)Thiazolylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+H)	654.3	654
32	Ms-(D)3-Pyridylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+H)	648.3	648
33	N-Boc-N-CH_3-(D)Phe-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+NH4)	700.4	700

34	Boc-(D)2-Pyridylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+H)	670.4	670
35	Ac-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+NH4)	481.3	481
36	Boc-(D)2-Thienylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+NH4)	692.4	692
37	H-(D)2-Pyridylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$ •HCl	NH_3/CI (M+H)	570.3	570
38	H-(D)2-Thienylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$•HCl	NH_3/CI (M+H)	575.3	575
39	Ms-(D)2-Pyridylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+H)	648.3	648
40	Ms-(D)2-Thienylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+NH4)	670.3	670
41	(2-Pyrimidylthio)acetyl-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+H)	574.3	574
42	trans-3-(3-pyridyl)acryl-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+H)	553.3	553
43	(4-Pyridylthio)acetyl-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+H)	573.3	573
44	Succinyl-(D)Phe-Pro-boroPhe-(m-CN)-OH	NH_3/CI EG ester+NH4	578.3	578
45	3-Pyridylpropionyl-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+H)	553.3	555
46	Boc-(D)Phe-Aze-boroPhe-(m-CN)-$C_{10}H_{16}$	NH_3/CI (M+NH4)	672.4	672
47	H-(D)Phe-Aze-boroPhe-(m-CN)-$C_{10}H_{16}$•HCl	NH_3/CI (M+H)	555.3	555
48	Hydrocinnamoyl-Pro-boroOrn(CH=NH)]OH•BSA	FAB EG ester+H	445.5	445
49	Hydrocinnamoyl-Pro-boroIrg($CH_2CH=CH_2$)-OH• HBr	ESI (M+H)	461	461
50	Hydrocinnamoyl-Pro-boroIrg(CH_3)-OH • HBr	ESI (M+H)	435	435

51	Cbz-(D)Phe-Pro-boroIrg(CH_3)-$C_{10}H_{16}$ • HBr	NH_3/CI (M+H)	718	718
52	Ac-(D)Phe-Pro-boroIrg(CH_3)-OH • HBr	ESI (M+H)	492	492
53	Hydrocinnamoyl-Pro-boroIrg(CH_2CH_3)-OH • HBr	ESI (M+H)	449	449
54	Ac-(D)Phe-Pro-boroArg(CH_3)-OH • HCl	NH_3/CI EG ester+H	501	501
55	Hydrocinnamoyl-Pro-boroArg(CH_3)-OH • HCl	ESI (M+H)	418	418
56	Ms-(D)Phe-Pro-boroArg(CH_3)-OH• HCl	ESI (M+H)	511	511
57	Ms-(D)Phe-Pro-boroOrn(CH=NH)-OH • HCl	ESI (M+H)	482	482
58	$PhSO_2$-(D)Phe-Pro-boroArg(CH_3)-OH • HCl	ESI (M+H)	573	573
59	$PhSO_2$-(D)Phe-Pro-boroOrn(CH=NH)-OH • HCl	ESI (M+H)	544	544
60	Ms-(D)Phe(4-fluoro)-Pro-boroOrn(CH=NH)-OH • HCl	ESI (M+H)	500	500
61	$PhCH_2SO_2$-(D)Phe-Pro-boroArg(CH_3)-OH • HCl	ESI (M+H)	587	587
62	$PhCH_2SO_2$-(D)Phe-Pro-boroOrn(CH=NH)-OH • HCl	ESI (M+H)	558	558
63	$CH_3CH_2CH_2SO_2$-(D)Phe-Pro-boroOrn(CH=NH)-OH • HCl	ESI (M+H)	510	5[illegible]
64	$CH_3CH_2CH_2SO_2$-(D)Phe-Pro-boroArg(CH_3)-OH • HCl	ESI (M+H)	539	539
65	$CH_3(CH_2)_3SO_2$-(D)Phe-Pro-boroArg(CH_3)-OH • HCl	ESI (M+H)	553	553
66	$CH_3(CH_2)_3SO_2$-(D)Phe-Pro-boroOrn(CH=NH)-OH • HCl	ESI (M+H)	524	524
67	Ac-(D)Phe-Sar-boroOrn(CH=NH)-OH•HCl			
68	Ms-(D)Phe-Sar-boroOrn(CH=NH)-OH•H			

69	Phenethyl-SO_2-(D)Phe-Sar-boroOrn(CH=NH)-OH•HCl			
70	Boc-(D)Phe-Sar-boroOrn(CH=NH)-OH•HCl			
71	N-alpha-[boroOrn(CH=NH)-OH]-(2-trans benzylcarboxamido)-cyclopentane-1-carboxamide•HCl			
72	H-(D)Phe-Sar-boroOrn(CH=NH)-$C_{10}H_{16}$•2HCl			
73	Boc-(D)Phe-Sar-boroPhe(m-CN)-$C_{10}H_{16}$			
74	Boc-(D)Phe-Aze-boroOrn(CH=NH)-OH•HCl			
75	H-(D)Phe-Sar-boroPhe(m-CN)-$C_{10}H_{16}$•2HCl			
76	4-(Phenyl)benzoyl-boroOrn(CH=NH)-$C_{10}H_{16}$•HCl			
77	Z-(D)Phe-Pro-boroOrn(CH=NH)-OH•HCl	NH_3/Cl pinacol ester+H	620.58	620.3[illegible]
78	H-boroPhe-(p-CN)-$C_{10}H_{16}$•HCl			
79	Boc-(D)Phe-Pro-N(CH_3)CH[(CH_2)3NHC(NH)H]-B$(OH)_2$			
80	Boc-(D)Phe-Pro-N(Phenyl)CH[(CH_2)3NHC(NH)H]-B$(OH)_2$			
81	Boc-(D)Phe-Pro-N(benzyl)CH[(CH_2)3NHC(NH)H]-B$(OH)_2$			
82	Boc-(D)Phe-Pro-N(CH_3)CH[(CH_2)3NHC(NH)H]-B$(OMe)_2$			
83	Boc-(D)Phe-Pro-N(CH_3)CH[(CH_2)3NHC(NH)H]-B[N(Me)]$_2$			
84	Boc-(D)Phe-Pro-N(CH_3)CH[(CH_2)3NHC(NH)H]-B$(F)_2$			
85	FMoc-(D)Phe-Pro-NHCH[(CH_2)3NHC(NH)H]-B$(OC_{10}H_{16})_2$			

86 Ac-(D)cyclohexylalanyl-Pro-NHCH[$(CH_2)_3$NHC(NH)H]-B$(OC_{10}H_{16})_2$

87 Ac-(D)Phe-Gly-NHCH[$(CH_2)_3$NHC(NH)H]-B$(OC_{10}H_{16})_2$

88 Ac-(D)Phe-Pro-NHCH[$(CH_2)_3$NHC(NOH)NH_2]-B$(OC_{10}H_{16})_2$

91 Ac- (D)Phe-Pro-boroPhe-(p-Br)-$C_{10}H_{16}$

92 Ac- (D)Phe-Pro-boroPhe-(p-NH_2)-$C_{10}H_{16}$

93 Ac- (D)Phe-Pro-boroPhe-(p-NHC(NH)NH_2)-$C_{10}H_{16}$

95 Ac- (D)Phe-Pro-boroPhe-(p-CH_2NHC(NH)NH_2)-$C_{10}H_{16}$

96 Ac- (D)Phe-Pro-boroPhe-(m-CH_2NHC(NH)NH_2)-$C_{10}H_{16}$

97 Ac- (D)Phe-Pro-boroPhe-(m-CH_2NHC(NH)NHCN)-$C_{10}H_{16}$

98 Z-Leu-Ser(Ot-Bu)-Asn-Leu-Ser(Ot-Bu)-Asn-Leu-Ser(Ot-Bu)-Asn-Leu-Ser(Ot-Bu)-Asn-NHCH[$(CH_2)_3$NHC(NH)H]-B$(OC_{10}H_{16})_2$

99 H-Leu-Ser(Ot-Bu)-Asn-Leu-Ser(Ot-Bu)-Asn-Leu-Ser(Ot-Bu)-Asn-Leu-Ser(Ot-Bu)-Asn-NHCH[$(CH_2)_3$NHC(NH)H]-B$(OC_{10}H_{16})_2$

100 Z-Leu-Ser-Asn-Leu-Ser-Asn-Leu-Ser-Asn-Leu-Ser-Asn-NHCH[$(CH_2)_3$NHC(NH)H]-B$(OC_{10}H_{16})_2$

101 H-Leu-Ser-Asn-Leu-Ser-Asn-Leu-Ser-Asn-Leu-Ser-Asn-NHCH[$(CH_2)_3$NHC(NH)H]-B$(OC_{10}H_{16})_2$

Utility

N-Acyl and N-peptide boronic acids which are described in the present invention represent a novel class of potent, reversible inhibitors of trypsin-like enzymes. Trypsin-like enzymes are a group of proteases which hydrolyzed peptide bonds at basic residues liberating either a C-terminal arginyl or lysyl residue. Among these are enzymes of the blood coagulation and fibrinolytic system required for hemostasis. They are Factors II, X, VII, IX, XII, kallikrein, tissue plasminogen activators, urokinase-like plasminogen activator, and plasmin. Enzymes of the complement system, acrosin (required for fertilization), pancreatic trypsin are also in this group. Elevated levels of proteolysis by these proteases can result in disease states. For example, consumptive coagulopathy, a condition marked by a decrease in the blood levels of enzymes of both the coagulation system, the fibrinolytic system and accompanying protease inhibitors is often fatal. Intervention by a synthetic inhibitor would clearly be valuable. More specifically, proteolysis by thrombin is required for blood clotting. Inhibition of thrombin results in an effective inhibitor of blood clotting. The importance of an effective inhibitor of thrombin is underscored by the observation that conventional anticoagulants such as heparin (and its complex with the protein inhibitor, antithrombin III) are ineffective in blocking arterial thrombosis associated with myocardial infractions and other clotting disorders. However, a low molecular weight thrombin inhibitor, containing a different functionality, was effective in blocking arterial thrombosis [Hanson and Harker (1988) *Proc. Natl. Acad.*

Sci. U.S.A. **85**, 3184-3188]. Therefore, we have chosen to demonstrate utility of compounds in the inhibition of thrombin, both as in buffered solutions and in plasma. Specifically, the compounds have utility as drugs for the treatment of diseases arising from elevated thrombin activity such as myocardial infarction, and as reagents used as anticoagulants in the processing of blood to plasma for diagnostic and other commercial purposes.

When used in the processing of blood products, the compounds of this invention may be mixed with whole blood without the need for any additional anticoagulants. The compounds of this invention serve to inhibit blood coagulation thereby facilitating the processing of whole blood into desired cellular components or plasma proteins. Once the processing is complete, the compounds may be removed, if so desired, by membrane ultrafiltration, dialysis, or diafiltration to afford the desired product. The low molecular weight of these compounds relative to conventional anticoagulants like heparin allow them to be separated from desired products more easily.

Compounds of the present invention are expected to be effective in the control of aberrant proteolysis and a number of accompanying disease states such as inflammation, pancretitis, and heritary angioedema.

The in vitro effectiveness of compounds of the present invention as inhibitors of the blood coagulation protease thrombin was determined under two different conditions: (1) measurements in buffered solutions using a synthetic substrate; (2) measurement in plasma where the rate of blood clotting is determined. For the former, the chromogenic substrate S2238 (H-(D)Phe-Pip-Arg-PNA, where PIP refers to pipecolic acid; Helena Laboratories, Beaumont, TX) was used following procedures similar to those described in Kettner et al. *J. Biol. Chem.* **265** 18289-18297 (1990). Here hydrolysis

resulted in the release of pNA which was monitored spectrophotometricaly by measuring the increase in absorbance at 405 nm. The Michaelis constant, K_m, for substrate hydrolysis was determined at 25 °C in 0.10 M sodium phosphate buffer, pH 7.5, containing 0.20 M NaCl, and 0.5 % PEG 8000 using the method of Lineweaver and Burk.

Values of K_i were determined by allowing thrombin (0.19 nM) to react with substrate (0.20 mM) in the presence of inhibitor. Reactions were allowed to go for 30 minutes and the velocities (rate of absorbance change vs time) were measured in the time frame of 25-30 minutes. The following relationship was used to calculate K_i values.

$$\frac{v_o - v_s}{v_s} = \frac{I}{K_i(1 + S/K_m)}$$

where

v_o is the velocity of the control in the absence of inhibitor;

v_s is the velocity in the presence of inhibitor;

I is the concentration of inhibitor;

K_i is the dissociation constant of enzyme: inhibitor complex;

S is the concentration of substrate;

K_m is the Michaelis constant.

Inhibition of blood clotting activity of thrombin in plasma was determined in rabbit plasma. Plasma was prepared by diluting blood 1:10 with 3.2% aqueous citric acid and centrifuging. Buffer consisted of 0.10 M Tris, pH 7.4, containing 0.9% sodium chloride, and 2.5 mg/mL bovine serum albumin. Bovine thrombin was obtained from Sigma and was diluted to 24 NIH units/mL. Plasma (200 µL) and buffer (50 µL) containing inhibitor were incubated 3 min at 37 °C in a fibrameter. Reactions

were initiated by adding thrombin (50µL) and clotting times measured. Controls were run under identical conditions except in the absence of inhibitor. The final concentration of thrombin was 4 NIH units/mL.

Table 2 - Inhibition of Thrombin.

Ex #	K_i [a] (nM)
1	750
2	0.26
3	0.38
4	0.28
6	0.085
7	0.040
8	0.18
9	.05
11	3.2
12	2.8
13	4.83
14	10
15	40
16	134
17	0.27
20	0.14
23	0.55
24	0.059
27	0.17
28	0.37
32	0.48
34	0.33
36	0.381
40	0.19
46	0.55
50	<859
54	1

62	0.03
63	0.5
64	0.5
67	8.2
73	81
74	<0.5
76	110

a Ki values were measure at 25 °C at pH 7.5.

Another measure of compound effectiveness toward prolonging clotting times can be reported as IC50 (level of inhibitor required to prolong clotting to the time observed for 2 NIH units/mL thrombin in the absence of inhibitor). Representative of data for compounds of the present invention, Examples 3, 7, 9, 11, and 12 increased thrombin clotting times 2-fold at 0.25, <0.075, 0.10, 0.60, and 0.85 μM, respectively.

The effectiveness of compounds of the present invention as anticoagulants in vivo was demonstrated by the prolongation of the activated partial thromboplastin time of samples of blood taken from conscious dogs or anesthetized rats after either oral or intravenous administration at doses of the compounds from 0.5 to 10 mg/kg. Arterial or venous blood was withdrawn by syringe and mixed with 1/10 volume 3.2% sodium citrate. Plasma was obtained after centrifugation and a standard clinical activated partial thromboplastin time (APTT reagent, Sigma Chemical Co., St. Louis, Mo.) determined at 37°C in a fibrometer. Results from blood samples obtained at various times after dosing showed an effective anticoagulant response which was at least equivalent to doubling of activated partial thromboplastin time as compared to the value obtained prior to dosing. In this model, Examples 4, 57, and 77

were shown to be effective following i.v. dosing and Examples 4, 56, 57, 60, and 66 effective following oral dosing. Similarly, oral administration of Examples 31 and 54 resulted in at least a 2-fold elevation in anticoagulant activity in an identical model except activity was measured by increases in thrombin clotting times.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Sheng-Lian O. Lee
John Matthew Fevig
Charles Adrian Kettner
David L. Carini

(ii) TITLE OF INVENTION: Amidino and Guanidino Substituted Boronic Acid Inhibitors of Trypsin-Like Enzymes

(iii) NUMBER OF SEQUENCES: 4

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: The Du Pont Merck Pharmaceutical Company
(B) STREET: 1007 Market Street, Legal Department
(C) CITY: Wilmington
(D) STATE: DE
(E) COUNTRY: U.S.
(F) ZIP: 19898

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: 3.50 inch disk
(B) COMPUTER: Apple Macintosh
(C) OPERATING SYSTEM: Apple Macintosh
(D) SOFTWARE: Microsoft Word

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: 08/052,835
(B) FILING DATE: ___________
(C) CLASSIFICATION: unknown

(vii) PRIOR APPLICATION DATA: None

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Reinert, Norbert, F.
(B) REGISTRATION NUMBER: 18,926
(C) REFERENCE/DOCKET NUMBER: DM-6567-A

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 302-892-8867
(B) TELEFAX: 302-892-8536

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULAR TYPE: peptide
(vi) ORIGINAL SOURCE: synthetic
(ix) FEATURE:
(D) OTHER INFORMATION: Example Number 98 at page 36 and within Table 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
Xaa Xaa Asn Leu Xaa Asn Leu Xaa Asn Leu Xaa Asn
     1                5                  10
```

(2) INFORMATION FOR SEQ ID NO:2:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acids
(C) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Leu Xaa Asn Leu Xaa Asn Leu Xaa Asn Leu Xaa Asn
1 5 10

(3) INFORMATION FOR SEQ ID NO:3:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULAR TYPE: peptide
(vi) ORIGINAL SOURCE: synthetic
(ix) FEATURE:
(D) OTHER INFORMATION: Example Number 100 at page 36 and within Table 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Xaa Ser Asn Leu Ser Asn Leu Ser Asn Leu Ser Asn
1 5 10

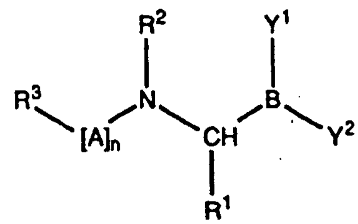
(3) INFORMATION FOR SEQ ID NO:4:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12
(B) TYPE: amino acids
(C) TOPOLOGY: linear
(ii) MOLECULAR TYPE: peptide
(vi) ORIGINAL SOURCE: synthetic
(ix) FEATURE:
(D) OTHER INFORMATION: Example Number 101 at page 36 and within Table 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Leu Ser Asn Leu Ser Asn Leu Ser Asn Leu Ser Asn
1 5 10

What is Claimed is:

1. A compound of formula (I)



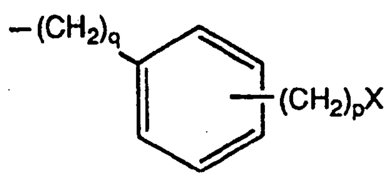
I

wherein

R^1 is

a) C1-C12-alkyl substituted with -CN, -C(NH)NHR^6, -NHC(NH)H, -NHC(NH)NHR^6, -SC(NH)NHR^6, -NHC(NH)NHOH, -NHC(NH)NHCN, -NHC(NH)NHCOR^6, or

b)



;

X is

a) halogen (F, Cl, Br, I)
b) -CN,
c) $-NO_2$,
d) $-CF_3$,
e) $-NH_2$
f) -NHC(NH)H,
g) -NHC(NH)NHOH,
h) -NHC(NH)NHCN,
i) -NHC(NH)NHR^6,
j) -NHC(NH)NHCOR^6,
k) -C(NH)NHR^6,
l) -C(NH)NHCOR^6,
m) -C(O)NHR^2,
n) $-CO_2R^2$,

o) $-OR^2$, or

p) $-OCF_3$

q) $-SC(NH)NHR^6$,;

R^2 is

a) H,

b) C1-C4-alkyl,

c) aryl, wherein aryl is phenyl or napthyl optionally substituted with one or two substituents selected from the group consisting of halo (F, Cl, Br, I), C1-C4-alkyl, C1-C4-alkoxy, $-NO_2$, $-CF_3$, $-S(O)_r$-C1-C4-alkyl, -OH, $-NH_2$, -NH(C1-C4-alkyl), -N(C1-C4-alkyl)$_2$, $-CO_2R^4$, or

d) -C1-C4-alkylaryl, where aryl is defined above;

R^3 is H, alkyl, aryl, alkylaryl or an NH_2-blocking group comprised of 1-20 carbon atoms;

R^4 and R^5 are independently

a) H,

b) C1-C4-alkyl, or

c) $-CH_2$-aryl, where aryl is defined above;

R^6 is

a) H,

b) C1-C4-alkyl,

c) aryl, wherein aryl is phenyl or napthyl optionally substituted with one or two substituents selected from the group consisting of halo (F, Cl, Br, I), C1-C4-alkyl, C1-C7-alkoxy, $-NO_2$, $-CF_3$, $-S(O)_r$-C1-C4-alkyl, -OH, $-NH_2$, -NH(C1-C4-alkyl), -N(C1-C4-alkyl)$_2$, $-CO_2R^4$, or

d) -C1-C4-alkylaryl, where aryl is defined above;

A is an amino acid residue or a peptide comprised of 2-20 amino acid residues;

Y^1 and Y^2 are

a) -OH,

b) -F,

c) C1-C8-alkoxy, or

when taken together Y^1 and Y^2 form a

d) cyclic boron ester where said chain or ring contains from 2 to 20 carbon atoms and, optionally, 1-3 heteroatoms which can be N, S, or O,

n is 0 or 1;

p is 0 to 3;

q is 0 to 4;

r is 0 to 2;

and pharmaceutically acceptable salts thereof, with the proviso that when R^1 is aliphatic, an R^6 subsitiuent on $-NHC(NH)NHR^6$ cannot be H.

2. A compound of claim 1 where

Y^1 and Y^2 are

a) -OH,

when taken together Y^1 and Y^2 form a

b) cyclic boron pinacol ester, or

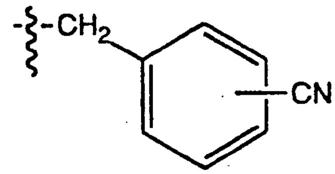
c) cyclic boron pinanediol ester;

R^1 is

a) $-(CH_2)_3NHC(NH)H$,

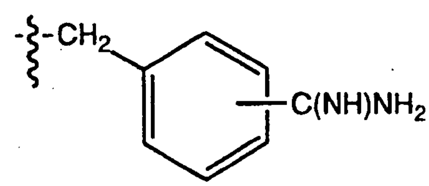
b) $-(CH_2)_4C(NH)NH_2$,

c)



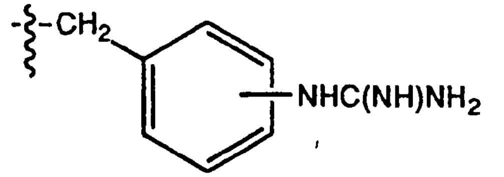
,

d)



, or

e)



R^2 is H;

A is Pro or (D)Phe-Pro;

R^3 is

a) H,

b) Boc,

c) Z,

d) Ac,

e) hydrocinnamoyl,

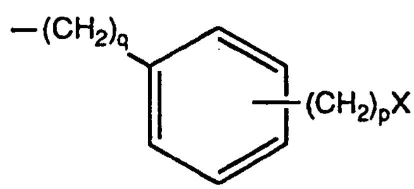
f) C1-C10 alkyl sulfonyl, or

g) C1-C15 alkylaryl sulfonyl.

3. A compound of claim 1 where

R^1 is

a)



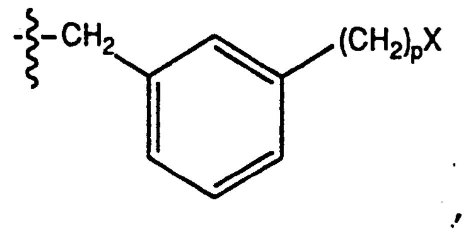
4. A compound of claim 1 where

R^1 is

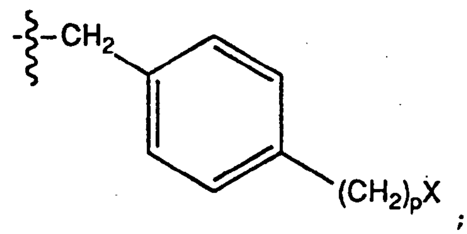
a) C3-C4-alkyl substituted with -CN, $-C(NH)NH_2$, -NH-C(NH)H.

5. A compound of claim 1 where
R^1 is

a)



b)



X is

a) halogen (Cl, Br)
b) -CN,
c) $-C(NH)NH_2$,
d) $-NH_2$
e) $-NHC(NH)NH_2$;

p is 0 to 1.

6. A compound of claim 1 where R^3 is H, alkyl, Ac, Boc, C1-C10 alkyl sulfonyl, C1-C15 alkylaryl sulfonyl, C1-C15 aryl sulfonyl.

7. A compound of claim 1 where n is 0.

8. A compound of claim 1 where [A] is comprised independently of amino acid residues in the D or L configuration selected from the group consisting of Ala, Arg, Asn, Asp, Aze, Cys, Gln, Glu, Gly, His, HomoLys, Ile, Leu, Lys, Met, Orn, Phe, Phe(4-fluoro), Pro, Ser, Thr, Trp, Tyr, and Val.

9. A compound of claim 1 where [A] is comprised of either Pro or (D)Phe-Pro.

10. A compound of claim 1 selected from the group:

- Ac-(D)Phe-Pro-NH-CH[$(CH_2)_4CN$]BO_2-$C_{10}H_{16}$

- Ac-(D)Phe-Pro-NHCH[$(CH_2)_4$C(NH)NH_2]BO_2-$C_{10}H_{16}$
- Ac-(D)Phe-Pro-NHCH[$(CH_2)_3$-NHC(NH)H]$B(OH)_2$
- Boc-(D)Phe-Pro-NHCH[$(CH_2)_3$-NHC(NH)H]$B(OH)_2$.
- Ac-(D)Phe-Pro-boroPhe[*m*-C(NH)NH_2]-$C_{10}H_{16}$
- Ac-(D)Phe-Pro-boroPhe(*m*-CH_2NH_2)-$C_{10}H_{16}$
- Ac-(D)Phe-Pro-boroPhe(*m*-Br)-$C_{10}H_{16}$
- Ac-(D)Phe-Pro-boroArg(CN)-$C_{10}H_{16}$
- Ac-(D)Phe-Pro-boroPhe(*p*-CN)-$C_{10}H_{16}$
- Boc-(D)Phe-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- N,N-$(CH_3)_2$-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl (ISOMER I)
- Ac-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl
- Ms-(D)Phe-Pro-boroPhe-(m-CN)-OH•HCl
- Boc-(D)Thiazolylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- Boc-(D)3-Pyridylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- Ms-(D)3-Pyridylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- Boc-(D)2-Pyridylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- Boc-(D)2-Thienylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- Ms-(D)2-Thienylalanine-Pro-boroPhe-(m-CN)-$C_{10}H_{16}$
- Boc-(D)Phe-Aze-boroPhe-(m-CN)-$C_{10}H_{16}$
- Hydrocinnamoyl-Pro-boroIrg(CH_3)-OH•HBr
- Ac-(D)Phe-Pro-boroArg(CH_3)-OH•HCl
- $PhCH_2SO_2$-(D)Phe-Pro-boroOrn(CH=NH)-OH•HCl
- $CH_3CH_2CH_2SO_2$-(D)Phe-Pro-boroOrn(CH=NH)-OH•HCl
- $CH_3CH_2CH_2SO_2$-(D)Phe-Pro-boroArg(CH_3)-OH•HCl
- Ac-(D)Phe-Sar-boroOrn(CH=NH)-OH•HCl
- Boc-(D)Phe-Sar-boroPhe(mCN)-$C_{10}H_{16}$
- Boc-(D)Phe-Aze-boroOrn(CH=NH)-OH•HCl
- 4-(Phenyl)benzoyl-boroOrn(CH=NH)-$C_{10}H_{16}$•HCl

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/04058

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K 37/02
US CL :514/18
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/18

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS Online, APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,499,082 (SHENVI ET AL.) 12 February 1985, see entire document.	1-10
A	US A, 4,537,773 (SHENVI) 27 August 1985, see entire document.	1-10

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
08 AUGUST 1994	AUG 18 1994
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer DAVID LUKTON Jill Warden for Telephone No. (703) 308-0196

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